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(54) Title: STEM CELL LIBRARIES

(57) Abstract: A stem cell library is created by genetically modifying stem cells with nucleic acids encoding polypeptides which can promote stem cell differentiation into specific cell types. Alternatively, the stem cell library is exposed to an externally added factor that promotes stem cell differentiation into a desired cell line, e.g., neuronal or muscle. The library is used to determine the effect of the encoded protein on the differentiation process. The library is also used to produce nucleic acids for insertion into embryonic stem cells to produce transfected embryonic stem cells. The nucleic acids are inserted into a locus that permits widespread expression of the encoded polypeptide in animals produced from blastocysts that incorporate the transfected cells. Non-human chimeric animals produced by combining blastocysts derived from animal models of human disease and embryonic stem cells transfected with molecules from the library provide an *in vivo* system for therapeutic design.

AMENDED CLAIMS
received by the International Bureau on 26 July 2004 (26.07.04): claims 1 to 237 have been replaced by claims 1 to 242.

What is claimed is:

1. A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,
   (a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed into each of the cell types;
   (b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof; and
   (c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.

2. A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,
   (a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types;
   (b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof; and
   (c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.

3. A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,
   (a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus

whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types;

(b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

(c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.

4. The modified stem cell of any of claims 1, 2 or 3, wherein the stem cell is selected from an embryonic stem cell or an adult stem cell.

5. The modified stem cell of any of claims 1, 2, or 3, wherein the stem cell is an animal stem cell.

6. The modified stem cell of claim 5, wherein the animal stem cell is a mouse stem cell.

7. The modified stem cell of claim 5, wherein the animal stem cell is a human stem cell.

8. The modified stem cell of claim 6, wherein the animal stem cell is a mouse embryonic stem cell.

9. The modified stem cell of any of claims 1, 2, or 3, wherein the first locus is selected from ROSA26, ROSA5, ROSA11, and G3BP(BT5).

10. The modified stem cell of claim 9, wherein the first locus is ROSA26.

11. The modified stem cell of claim 1, wherein the first polypeptide is selected from one or more growth factors, differentiation factors, anti-differentiation factors, colony stimulating factors, cytokines, lymphokines, anti-inflammatory molecules, apoptotic and other anti-cancer molecules, anti-apoptotic molecules, proteins involved in signaling pathways, antibodies, and active fragments thereof.

12. The modified stem cell of claim 11, wherein the first polypeptide is a protein involved in a signaling pathway, and the signaling pathway is a Wnt pathway.

13. The modified stem cell of either of claims 1 or 2, wherein the first polypeptide is selected from a ligand and a receptor.

14. The modified stem cell of claim 13, wherein the ligand is a Wnt ligand and the receptor is a Wnt receptor.

15. The modified stem cell of either of claims 1 or 2, wherein the first heterologous nucleic acid molecule encodes a human protein or an active fragment thereof.

16. The modified stem cell of claim 3, wherein the stem cell further comprises an episomal vector.

17. The modified stem cell of claim 16, wherein the episomal maintenance molecule is a polyoma large T antigen when the episomal vector comprises a polyoma origin of replication.

18. The modified stem cell of claim 16, wherein the episomal vector comprises a second heterologous nucleic acid molecule.

19. The modified stem cell of claim 18, wherein the second heterologous nucleic acid molecule encodes a second polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

20. The modified stem cell of claim 18, wherein the second heterologous nucleic acid molecule encodes a second polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, and ubiquitin E3 ligases.

21. The modified stem cell of claim 18, wherein the second nucleic acid molecule is an RNAi molecule.

22. The modified stem cell of claim 16, wherein the episomal vector comprises an origin of replication.

23. The modified stem cell of claim 22, wherein the origin of replication is a mutant enhancer ori from PyF101.

24. The modified stem cell of claim 18, wherein the episomal vector further comprises a promoter that regulates the expression of the second heterologous nucleic acid molecule.

25. The modified stem cell of claim 24, wherein the promoter is a tissue-specific promoter.

26. The modified stem cell of claim 24, wherein the promoter is selected from an inducible promoter and a constitutive promoter.

27. The modified stem cell of claim 26, wherein the promoter is an inducible promoter.

28. The modified stem cell of claim 27, wherein the inducible promoter is a tetracycline-responsive promoter.

29. The modified stem cell of claim 27, wherein the modified stem cell further comprises a third heterologous nucleic acid molecule that is capable of activating the inducible promoter.

30. The modified stem cell of claim 29, wherein the third heterologous nucleic acid molecule is present in a vector cassette that also comprises the first heterologous nucleic acid molecule.

31. The modified stem cell of claim 29, wherein the third heterologous nucleic acid molecule encodes rtTA.

32. A modified blastocyst from a first animal that comprises a modified stem cell from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

33. A modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the

modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, and ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

34. A modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

35. The modified blastocyst of any one of claims 32, 33, and 34, wherein the first animal and the second animal are the same or different species, provided that if the first animal is human, the second animal is also human.

36. The modified blastocyst of any one of claims 32, 33, and 34, wherein the stem cell is selected from an embryonic stem cell and an adult stem cell.

37. The modified blastocyst of any one of claims 32, 33, and 34, wherein the first and second animals are mice.

38. The modified blastocyst of claim 34, wherein the modified stem cell further comprises an episomal vector.

39. The modified blastocyst of claim 38, wherein the episomal maintenance factor is a polyoma large T antigen when the episomal vector comprises a polyoma origin of replication.

40. The modified blastocyst of claim 38, wherein the episomal vector comprises a second heterologous nucleic acid molecule.

41. The modified blastocyst of claim 40, wherein the second nucleic acid molecule encodes a second polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

42. The modified blastocyst of claim 40, wherein the second nucleic acid molecule encodes a second polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof.

43. The modified blastocyst of claim 40, wherein the second nucleic acid molecule is an RNAi molecule.

44. The modified blastocyst of claim 38, wherein the episomal vector further comprises an origin of replication.

45. The modified blastocyst of claim 44, wherein the origin of replication is a mutant enhancer ori from PyF101.

46. The modified blastocyst of claim 40, wherein the episomal vector further comprises a promoter that regulates the expression of the second heterologous nucleic acid molecule.

47. The modified blastocyst of claim 46, wherein the promoter is a tissue-specific promoter.

48. The modified blastocyst of claim 46, wherein the promoter is selected from an inducible promoter and a constitutive promoter.

49. The modified blastocyst of claim 46, wherein the promoter is an inducible promoter.

50. The modified blastocyst of claim 49, wherein the inducible promoter is a tetracycline-responsive promoter.

51. The modified blastocyst of claim 49, wherein the modified stem cell further comprises a third heterologous nucleic acid molecule that is capable of activating the inducible promoter.

52. The modified blastocyst of claim 51, wherein the third heterologous nucleic acid molecule is present in a vector cassette that also comprises the first heterologous nucleic acid molecule.

53. The modified blastocyst of claim 51, wherein the third heterologous nucleic acid molecule encodes rtTA.

54. A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

55. A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified term cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases,

kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

56. A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

57. The non-human chimeric animal of claim 56, wherein the modified stem cell further comprises an episomal vector.

58. The non-human chimeric animal of claim 57, wherein the episomal vector comprises a second nucleic acid molecule that encodes a second polypeptide.

59. The non-human chimeric animal of claim 58, wherein the episomal vector comprises an origin of replication.

60. The non-human chimeric animal of claim 59, wherein the origin of replication comprises that of a polyoma virus.

61. The non-human chimeric animal of claim 60, wherein the origin of replication is a mutant enhancer ori from PyF101.

62. The non-human chimeric animal of claim 61, wherein the episomal maintenance molecule is a polyoma large T antigen.

63. The non-human chimeric animal of claim 58, wherein the second polypeptide is selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

64. The non-human chimeric animal of claim 58, wherein the second polypeptide is selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof.

65. A tissue obtained from the non-human chimeric animal of any one of claims 54, 55, or 56.

66. The tissue of claim 65, selected from heart, lung, kidney, liver, brain, bone marrow, blood, bone, cartilage, prostate, ovary, skin, spinal cord, thymus, spleen, muscle, stomach, intestine, and pancreas.

67. A cell derived from the tissue of claim 65.

68. A cell obtained from the non-human chimeric animal of any one of claims 54, 55, and 56, wherein the cell is selected from heart cells, lung cells, kidney cells, liver cells, brain cells, bone marrow cells, blood cells, bone cells, cartilage cells, prostate cells, ovary cells, skin cells, spinal cord cells, thymus cells, spleen cells, muscle cells, stomach cells, intestinal cells, and pancreatic cells.

69. The non-human chimeric animal of any of claims 54, 55, and 56, wherein the blastocyst is a blastocyst of an animal model of a human disease, disorder, syndrome, or condition.

70. The non-human chimeric animal of claim 69, wherein the disease, disorder, syndrome, or condition is selected from an immune system disease, disorder, syndrome, or condition, a metabolic system disease, disorder, syndrome, or condition, a central nervous system disease, disorder, syndrome, or condition, and cancer.

71. The non-human chimeric animal of claim 69, wherein the animal model of a human disease, disorder, syndrome, or condition is selected from a SCID mouse, a NOD mouse, a knockout mouse, a Rb -/- mouse, a p53 -/- mouse, a mouse that over-expresses human Aβ, and a mouse that over-expresses TGFβ.

72. A differentiated cell, wherein the cell differentiates from the modified stem cell of any one of claims 1, 2, or 3.

73. The differentiated cell of claim 72, wherein the cell is selected from a heart cell, a lung cell, a kidney cell, a liver cell, a neuronal cell, a bone marrow cell, a blood cell, a bone cell, a cartilage cell, a prostate cell, an ovarian cell, a skin cell, a thymus cell, a spleen cell, a muscle cell, a fibroblast cell, a stomach cell, an intestinal cell, a pancreatic cell, and a precursor thereof.

74. The differentiated cell of claim 72, wherein the cell is selected from a lymphocyte, a dendritic cell, a macrophage, a natural killer cell, a neutrophil, an eosinophil, a basophil, a megakaryocyte, an erythrocyte, and a precursor thereof.

75. A non-human transgenic animal that is produced from a cross between two chimeric animals of any one of claims 54, 55, or 56, or a progeny thereof wherein the transgenic animal is homozygous for the first heterologous nucleic acid molecule.

76. A composition comprising a plurality of the modified stem cells of any of claims 1, 2, or 3.

77. A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

78. A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

79. A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

80. A method of making a modified blastocyst, comprising the steps of:

(a) obtaining a blastocyst from a first animal;

(b) obtaining a modified stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase, and

(c) introducing the modified stem cell into the blastocyst to produce the modified blastocyst.

81. A method of making a modified blastocyst, comprising the steps of:

(a) obtaining a blastocyst from a first animal;

(b) obtaining a modified stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase; and

(c) introducing the modified stem cell into the blastocyst to produce the modified blastocyst.

82. A method of making a modified blastocyst, comprising the steps of:

(a) obtaining a blastocyst from a first animal;

(b) obtaining a modified stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an a episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase; and

(c) introducing the modified stem cell into the blastocyst to produce the modified blastocyst.

83. The method of claim 82, wherein the method further comprises, before step (c), the step of introducing an episomal vector into the modified stem cell.

84. The method of claim 83, wherein the episomal vector comprises an origin of replication.

85. The method of claim 84, wherein the episomal vector further comprises a second heterologous nucleic acid molecule.

86. The method of claim 85, wherein the second heterologous nucleic acid molecule encodes a second polypeptide, wherein the second polypeptide is selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

87. The method of claim 85, wherein the second heterologous nucleic acid molecule encodes a second polypeptide, wherein the second polypeptide is selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof.

88. A method of making a non-human chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant animal; and

(c) allowing the blastocyst to develop into a non-human chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises modified stem cell from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the

modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof; and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

89. A method of making a non-human chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant non-human animal; and

(c) allowing the blastocyst to develop into a non-human chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises one or more modified stem cells from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

90. A method of making a non-human chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant non-human animal; and

(c) allowing the blastocyst to develop into a non-human chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises one or more modified stem cells from a second animal,

wherein the modified stem cell comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types;

the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

91. The method of claim 90, wherein the modified stem cell further comprises an episomal vector.

92. The method of claim 91, wherein the episomal maintenance molecule is a polyoma large T antigen when the episomal vector comprises a polyoma origin of replication.

93. The method of claim 91, wherein the episomal vector comprises a second heterologous nucleic acid molecule.

94. The method of claim 93, wherein the second heterologous nucleic acid molecule encodes a second polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

95. The method of claim 93, wherein the second nucleic acid molecule encodes a second polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof.

96. The method of claim 93, wherein the second nucleic acid molecule is an RNAi molecule.

97. The method of claim 91, wherein the episomal vector comprises an origin of replication.

98. The method of claim 97, wherein the origin of replication is a mutant enhancer ori from PyF101.

99. The method of claim 91, wherein the episomal vector further comprises a promoter that regulates the expression of the second heterologous nucleic acid molecule.

100. The method of claim 99, wherein the promoter is a tissue-specific promoter.

101. The method of claim 99, wherein the promoter is selected from an inducible promoter and a constitutive promoter.

102. The method of claim 99, wherein the promoter is an inducible promoter.

103. The method of claim 102, wherein the inducible promoter is a tetracycline-responsive promoter.

104. The method of claim 102, wherein the modified stem cell further comprises a third heterologous nucleic acid molecule that is capable of activating the inducible promoter.

105. The method of claim 104, wherein the third heterologous nucleic acid molecule is present in a vector cassette that also comprises the first heterologous nucleic acid molecule.

106. The method of claim 104, wherein the third heterologous nucleic acid molecule encodes rtTA.

107. A method of determining an *in vivo* effect of a first polypeptide in an animal, comprising the steps of:

(a) obtaining a non-human chimeric animal of any one of claims 54-64; and

(b) observing the non-human chimeric animal for phenotypic, histologic, or physiologic changes.

108. A method of determining an *in vitro* effect of a first polypeptide on a cell, comprising the steps of:

(a) obtaining a modified stem cell of claim 1; and

(b) observing the modified stem cell for phenotypic, histologic, or physiologic changes.

109. A method of determining an *in vitro* and/or *in vivo* effect of a first polypeptide in an animal comprising the steps of:

(a) obtaining a composition comprising a plurality of modified stem cells of claim 1;

(b) observing the modified stem cells for phenotypic, histologic, or physiologic changes;

(c) introducing at least one modified stem cell into a blastocyst to produce a modified blastocyst;

(d) implanting the modified blastocyst into a pseudopregnant non-human animal;

(e) allowing birth of a non-human chimeric animal that developed from a modified blastocyst; and

(f) observing the non-human chimeric animal for phenotypic, histologic, or physiologic changes.

110. A method for production of a heterologous polypeptide comprising the steps of:

(a) obtaining a modified stem cell of claim 1; and

(b) allowing the modified stem cell to proliferate whereby, the heterologous polypeptide is produced.

111. The method of claim 110, wherein the heterologous nucleic acid molecule of the modified stem cell is under regulatory control of a first promoter, wherein the first promoter is inducible, comprising the step of activating the inducible promoter.

112. The method of claim 110, wherein the heterologous polypeptide is a transmembrane protein, and the modified stem cell expresses the transmembrane protein on its cell surface.

113. The method of claim 110, wherein the heterologous polypeptide is a secreted protein, and the modified stem cell secretes the secreted protein into a growth medium.

114. The method of claim 110, wherein the heterologous polypeptide is an intracellular protein, and the modified stem cell expresses the intracellular protein intracellularly.

115. A library comprising a plurality of modified stem cells of claim 1, wherein the plurality of modified stem cells comprise modified stem cells, wherein the heterologous nucleic acid molecule encodes a first member of a family of proteins or an active fragment thereof, and second modified stem cells,

wherein the heterologous nucleic acid molecule encodes a second member of the family of proteins or an active fragment thereof.

116. The library of claim 115, wherein the library comprises at least 100, at least 200, at least 300 , at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, or at least 1000 modified stem cells, each comprising at least one different heterologous nucleic acid molecule of the same family of proteins.

117. The library of claim 115, wherein the library comprises at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, at least 1600, at least 1700, at least 1800, at least 1900, or at least 2000 modified stem cells, each comprising at least one different heterologous nucleic acid molecule of the same family of proteins.

118. The library of claim 115, wherein the family of proteins is selected from secreted proteins, single transmembrane proteins, multi-transmembrane proteins, extracellular domains of transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof.

119. The library of claim 115, wherein the family of proteins is selected from growth factors, differentiation factors, cytokines, lymphokines, Notch ligands, Notch receptors, Wnt ligands, Wnt receptors, anti-inflammatory factors, anti-apoptotic factors, apoptotic and other anti-cancer molecules, other signaling ligands and receptors, and active fragments thereof.

120. The library of claim 115, wherein the heterologous nucleic acid molecules are substantially equally represented in the library.

121. A composition comprising a first modified and at least a second modified stem cell,

wherein the first modified stem cell comprises at least a first heterologous nucleic acid molecule that encodes a first polypeptide, and the second modified stem cell comprises at least a second heterologous nucleic acid molecule that encodes a second polypeptide,

wherein the first polypeptide encodes a secreted factor and the second polypeptide encodes a receptor,

wherein the first nucleic acid integrates at a first locus of a chromosome of the first modified stem cell and the second nucleic acid integrates at a second locus of a chromosome of the second modified stem cell, and

wherein the first and second locus are identical.

122. The composition of claim 121, wherein the first locus is selected from ROSA26, ROSA5, ROSA11, and G3BP(BT5).

123. A composition comprising a first library of modified stem cells and a second library of modified stem cells,

wherein the first library of modified stem cells comprises a plurality of modified stem cells, wherein the plurality of modified stem cells comprises at least a first modified stem cell that is transfected with a first heterologous nucleic acid molecule that encodes a first member of a first family of proteins or an active fragment thereof and at least a second modified stem cell that is transfected with a second heterologous nucleic acid molecule that encodes a second member of the first family of proteins or an active fragment thereof,

wherein the second library of modified stem cells comprises a plurality of modified stem cells, wherein the plurality of modified stem cells comprises at least a first modified stem cell that is transfected with a first heterologous nucleic acid molecule that encodes a first member of a second family of proteins or an active fragment thereof and at least a second modified stem cell that is transfected with a second heterologous nucleic acid molecule that encodes a second member of the second family of proteins or an active fragment thereof, and

wherein the first family of proteins is a family of secreted proteins or extracellular domains of single transmembrane proteins, and the second family of proteins is a family of receptors.

124. A modified mesenchymal stem cell comprising at least one first heterologous nucleic acid sequence encoding at least one first therapeutic molecule for a disease, disorder, syndrome, or condition, wherein said sequence is other than an anti-cancer agent.

125. The modified mesenchymal stem cell of claim 124, wherein the disease, disorder, syndrome, or condition is other than a hyperproliferative disease, disorder, syndrome, or condition.

126. The modified mesenchymal stem cell of claim 124, wherein the first therapeutic molecule is secreted.

127. The modified mesenchymal stem cell of 124, wherein the first therapeutic molecule is a molecule chosen from a growth factor, an anti-inflammatory factor, an anti-apoptotic factor, a vasodilator, an anti-coagulant, insulin, an extracellular domain of a receptor, an immunoglobulin chain, a biologically active fragment of the molecule, and a fusion protein comprising the molecule, or biologically active fragment thereof.

128. The modified mesenchymal stem cell of claim 127, wherein the first therapeutic molecule is a fusion protein and the fusion protein is cleavable to release a biologically active molecule or biologically active fragment thereof.

129. The modified mesenchymal stem cell of claim 124, further comprising at least one second heterologous nucleic acid sequence encoding a second therapeutic molecule that functions in conjunction with the first therapeutic molecule.

130. The modified mesenchymal stem cell of claim 127, wherein the first therapeutic molecule is a growth factor selected from a factor that stimulates proliferation of a cell, a factor that stimulates differentiation of a cell, a factor that promotes change in the metabolic state of a cell, and a factor that promotes change in the gene expression pattern of a cell.

131. The modified mesenchymal stem cell of claim 127, wherein the change in the metabolic state of the cell comprises activation.

132. The modified mesenchymal stem cell of claim 124, wherein the first therapeutic molecule is a growth factor selected from KGF, PDGF, BDNF, erythropoietin, GM-CSF, and G-CSF.

133. The modified mesenchymal stem cell of claim 127, wherein the first therapeutic molecule is an anti-inflammatory factor.

134. The modified mesenchymal stem cell of claim 133, wherein the anti-inflammatory factor is an anti-TNF molecule.

135. The modified mesenchymal stem cell of claim 133, wherein the anti-inflammatory factor is an extracellular fragment of a receptor.

136. The modified mesenchymal stem cell of claim 124, wherein the cell is selected from a CNS cell, heart muscle cell, lung cell, kidney cell, bone marrow cell, lymphocyte, lymphocyte precursor, erythrocyte precursor, bone cell, cartilage

cell, pancreatic cell, liver cell, muscle cell, fibroblast, epithelial cell, and skin cell.

137. The modified mesenchymal stem cell of claim 124, further comprising at least one third heterologous nucleic acid sequence encoding a survival factor.

138. The modified mesenchymal stem cell of claim 137, wherein the survival factor is an anti-apoptotic factor.

139. The modified mesenchymal stem cell of claim 137, wherein the survival factor is Akt.

140. The modified mesenchymal stem cell of claim 137, wherein the survival factor is telomerase.

141. The modified mesenchymal stem cell of claim 124, wherein the first heterologous nucleic acid sequence is under the regulatory control of a promoter.

142. The modified mesenchymal stem cell of claim 141, wherein the promoter is an inducible promoter.

143. The modified mesenchymal stem cell of claim 141, wherein the promoter is a constitutive promoter.

144. The modified mesenchymal stem cell of claim 141, wherein the promoter is a tissue-specific promoter.

145. The modified mesenchymal stem cell of claim 141, wherein the promoter is heterologous to the first heterologous nucleic acid molecule.

146. The modified mesenchymal stem cell of claim 141, wherein the promoter is homologous to the first heterologous nucleic acid molecule.

147. A composition comprising the modified mesenchymal stem cell of claim 124 and a pharmaceutically acceptable carrier.

148. A kit comprising the composition of claim 147 and instructions for administration into an animal.

149. The kit of claim 148, wherein the animal is a human.

150. A modified mesenchymal stem cell comprising at least one first heterologous nucleic acid sequence and at least one second heterologous nucleic acid sequence, wherein the first heterologous sequence encodes one member of a dimeric molecule and the second heterologous nucleic acid encodes a second member of the dimeric molecule.

151. The modified mesenchymal stem cell of claim 150, wherein the dimeric molecule is a therapeutic molecule.

152. The modified mesenchymal stem cell of claim 150, wherein the dimeric molecule is an antibody.

153. The modified mesenchymal stem cell of claim 150, wherein the first heterologous nucleic acid sequence encodes an immunoglobulin heavy chain and the second heterologous nucleic acid sequence encodes an immunoglobulin light chain.

154. The modified mesenchymal stem cell of claim 152, wherein the antibody is an anti-inflammation antibody.

155. The modified mesenchymal stem cell of claim 152, wherein the antibody is an anti-cancer antibody.

156. A composition comprising the modified mesenchymal stem cell of claim 150 and a pharmaceutically acceptable carrier.

157. A kit comprising the composition of claim 156 and instructions for administration into an animal.

158. The kit of claim 157, wherein the animal is a human.

159. A modified mesenchymal stem cell comprising a mesenchymal stem cell that comprises at least one first heterologous nucleic acid sequence, wherein the first heterologous nucleic acid sequence encodes a therapeutic factor that is therapeutic for cancer and is other than a cytokine, a hormone, an extracellular matrix component, an enzyme, a signaling molecule, an anti-angiogenic polypeptide, an oncolytic virus, interferon-α, or interferon-β.

160. The modified mesenchymal stem cell of claim 159, wherein the therapeutic factor is an immunoglobulin.

161. The modified mesenchymal stem cell of claim 160, wherein the therapeutic factor is a single chain antibody.

162. A method for treatment of a disease, disorder, syndrome, or condition in a subject comprising the steps of:

(a) providing a modified mesenchymal stem cell of claim 1; and

(b) administering the modified mesenchymal stem cell to the subject,

wherein the disease, disorder, syndrome, or condition is other than cancer.

163. The method of claim 162, wherein the disease, disorder, syndrome, or condition is other than a hyperproliferative disease, disorder, syndrome, or condition.

164. The method of claim 162, wherein the disease, disorder, syndrome, or condition is inflammation and the first therapeutic molecule is an anti-inflammatory factor.

165. The method of claim 162, wherein the disease, disorder, syndrome, or condition is ischemic heart disease and the first therapeutic molecule is a vasodilator.

166. The method of claim 162, wherein the disease, disorder, syndrome, or condition is thrombosis and the first therapeutic molecule is an anti-coagulant.

167. The method of claim 162, wherein the disease, disorder, syndrome, or condition is diabetes and the first therapeutic molecule is insulin.

168. The method of claim 162, wherein the disease, disorder, syndrome, or condition is immunological, and the first therapeutic molecule is immunosuppressive.

169. The method of claim 162, wherein the disease, disorder, syndrome, or condition is immunological, and the first therapeutic molecule enhances the immune response of the subject.

170. A method for treatment of a hyperproliferative disease, disorder, syndrome, or condition in a subject comprising the steps of:

(a) providing a modified mesenchymal stem cell of 124; and

(b) administering the modified mesenchymal stem cell to the subject, wherein the first therapeutic molecule encoded by the first heterologous nucleic acid sequence is an antibody.

171. The method of claim 170, wherein the antibody is a single chain antibody.

172. A method for treatment of a disease, disorder, syndrome, or condition in a subject comprising the steps of:

(a) providing a modified mesenchymal stem cell of claim 130; and

(b) administering the modified mesenchymal stem cell to the subject.

173. The method of claim 172, wherein the disease, disorder, syndrome, or condition is a hyperproliferative disease, disorder, syndrome, or condition.

174. The method of claim 173, wherein the hyperproliferative disease, disorder, syndrome, or condition is cancer.

175. The method of claim 173, wherein the hyperproliferative disease, disorder, syndrome, or condition is psoriasis.

176. A chimeric non-human animal stem cell comprising a non-human animal stem cell and at least one first heterologous nucleic acid sequence, wherein the first heterologous nucleic acid sequence encodes a first human polypeptide other than β-galactosidase, wherein the first heterologous nucleic acid sequence is inserted at a first locus of a chromosome of the non-human animal, and wherein insertion of the first heterologous nucleic acid sequence at the first locus enables expression of the polypeptide in the chimeric stem cell in both a differentiated and undifferentiated state.

177. The chimeric non-human animal stem cell of claim 176, wherein the non-human animal stem cell is selected from an embryonic stem cell and an adult stem cell.

178. The chimeric non-human animal stem cell of claim 176, wherein the first locus is the ROSA26 locus.

179. The chimeric non-human animal stem cell of claim 176, wherein the first human polypeptide is a human secreted polypeptide.

180. The chimeric non-human animal stem cell of claim 179, wherein the secreted polypeptide is selected from a growth factor, differentiation factor, anti-differentiation factor, co-factor, cytokine, lymphokine, anti-inflammatory polypeptide, anti-cancer polypeptide, and apoptotic polypeptide.

181. The chimeric non-human animal stem cell of claim 179, wherein the human polypeptide is selected from a human transmembrane polypeptide, a human kinase, a human protease, a human phosphatase, a human phosphodiesterase, a human kinesin, a human hormone receptor, and a human ubiquitin ligase.

182. The chimeric non-human animal stem cell of claim 179, wherein the first heterologous nucleic acid sequence is under regulatory control of a first promoter.

183. The chimeric non-human animal stem cell of claim 182, wherein the first promoter is a tissue-specific promoter.

184. The chimeric non-human animal stem cell of claim 182, wherein the first promoter is an inducible promoter.

185. The chimeric non-human animal stem cell of claim 183, wherein the tissue-specific promoter is specific for expression in one or more organs selected

from liver, lung, heart, kidney, skin, pancreas, brain, blood, bone marrow, gastrointestinal tract, breast, prostate, ovary, bone, and cartilage.

186. The chimeric non-human animal stem cell of claim 179, wherein the stem cell is differentiated.

187. The chimeric non-human animal stem cell of claim 186, wherein the stem cell is differentiated into a cell selected from a liver cell, a heart cell, a fat cell, a skin cell, a brain cell, a kidney cell, a lung cell, a hematopoietic cell, a pancreatic cell, an epithelial cell, a bone cell, a cartilage cell, and a precursor thereof.

188. The chimeric non-human animal stem cell of claim 187, wherein the stem cell is differentiated into a hematopoietic cell, and the hematopoietic cell is selected from a precursor of a red blood cell, a lymphocyte, a macrophage, a natural killer (NK) cell, and a neutrophil.

189. The chimeric non-human animal stem cell of claim 176, wherein the chimeric non-human animal stem cell comprises at least one second heterologous nucleic acid sequence, wherein the second heterologous nucleic acid sequence encodes a second polypeptide.

190. The chimeric non-human animal stem cell of claim 189, wherein the second polypeptide interacts with the first polypeptide.

191. The chimeric non-human animal stem cell of claim 190, wherein the second polypeptide is a secreted polypeptide.

192. The chimeric non-human animal stem cell of claim 189, wherein the second polypeptide is under regulatory control of a promoter.

193. The chimeric non-human animal stem cell of claim 192, wherein the second promoter is the same as the promoter of claim 182.

194. The chimeric non-human animal stem cell of claim 192, wherein the second promoter is different from the promoter of claim 182.

195. The chimeric non-human animal stem cell of claim 176, wherein the non-human animal is an animal used in pharmaceutical research.

196. The chimeric non-human animal stem cell of claim 176, wherein the non-human animal is a rodent.

197. The chimeric non-human animal stem cell of claim 196, wherein the rodent is a mouse.

198. A chimeric non-human blastocyst comprising at least one chimeric non-human animal stem cell,
wherein the chimeric non-human animal stem cell comprises a non-human animal stem cell and at least one first heterologous nucleic acid sequence,
wherein the first heterologous nucleic acid sequence encodes a first human polypeptide;
wherein the first heterologous nucleic acid sequence is inserted a first locus of a chromosome of the non-human animal stem cell; and
wherein insertion of the first heterologous nucleic acid sequence at the first locus enables expression of the polypeptide in the chimeric stem cell.

199. The chimeric non-human blastocyst of claim 198, wherein the blastocyst comprises a plurality of chimeric non-human animal stem cells.

200. The chimeric non-human blastocyst of claim 198, wherein the blastocyst is selected from a 2 cell stage blastocyst, a 4 cell stage blastocyst, an 8 cell stage blastocyst, a 16 cell stage blastocyst, a 32 cell stage blastocyst, a 64 cell stage blastocyst, and a 128 cell stage blastocyst.

201. The chimeric non-human blastocyst of claim 198, wherein the blastocyst is at least a 64 cell stage blastocyst.

202. A chimeric non-human animal produced from the chimeric non-human animal stem cell of claim 124, or the chimeric non-human blastocyst of claim 198, or a progeny thereof.

203. The chimeric non-human animal of claim 202, wherein the animal is used in pharmaceutical research.

204. The chimeric non-human animal of claim 202, wherein the animal is a rodent.

205. The chimeric non-human animal of claim 204, wherein the rodent is a mouse.

206. The chimeric non-human animal of claim 202, wherein the animal is treated to model human disease.

207. One or more cells derived from the animal of claim 202.

208. A non-human animal comprising at least one first heterologous polynucleotide that encodes a first heterologous polypeptide, wherein the animal is homozygous with respect to the first heterologous nucleic acid sequence, and the animal is produced from the chimeric non-human animal of claim 202 or a progeny thereof.

209. The non-human animal of claim 208, wherein the animal is produced by breeding said chimeric non-human animal or a progeny thereof.

210. The non-human animal of claim 209, wherein the chimeric non-human animal is bred by crossing one chimeric non-human animal with another chimeric non-human animal or a progeny thereof.

211. The non-human animal of claim 208, wherein the animal is treated to model human disease.

212. One or more cells derived from the animal of claim 208.

213. A chimeric non-human animal resulting from a cross between at least one first animal that is a chimeric non-human animal of claim 202 or a progeny thereof, or a first non-human animal comprising a first heterologous nucleic acid sequence that encodes a first heterologous polypeptide, wherein the animal is homozygous with respect to the first heterologous polynucleotide, and the animal is produced from a chimeric non human animal or a progeny thereof; and a second animal that is a non-human animal or a progeny of said second animal.

214. One or more cells derived from the animal of claim 213.

215. The non-human animal of claim 213, wherein the second animal provides an animal model of disease.

216. The non-human animal of claim 215, wherein the disease is selected from immune system disease, metabolic disease, central nervous system disease, and cancer.

217. The non-human animal of claim 216, wherein the second animal is selected from a SCID mouse, a NOD mouse, a knockout mouse, an Rb -/- mouse, a p53 -/- mouse, a mouse that over-expresses human amyloid β-peptide, and a mouse that over-expresses TGFβ.

218. The non-human animal of claim 217, wherein the animal is treated to model human disease.

219. One or more cells derived from the animal of claim 218.

220. The chimeric non-human animal of claim 218, wherein the human disease is selected from immune system disease, metabolic disease, central nervous system disease, and cancer.

221. The chimeric non-human animal of claim 218, wherein the disease is diabetes or Alzheimer's disease.

222. The non-human animal of claim 202 in which a functional gene has been replaced by a nonfunctional form of the gene, and the function of said gene is eliminated in whole or in part.

223. The non-human animal of claim 202, wherein the animal is a mouse.

224. The non-human animal of claim 202, wherein the non-human animal suffers from a disease, disorder, syndrome, or condition selected from abnormal growth, hormonal imbalance, early aging, abnormal bone formation, immune disorder disease, cancer, diabetes, skin disease, and CNS disease.

225. Isolated tissues derived from the non-human animal of claim 202.

226. One or more cells derived from the non-human animal of claim 202.

227. One or more cells of claim 208, wherein the cells are selected from stem cells, bone marrow cells, liver cells, pancreatic cells, skin cells, hematopoietic cells, kidney cells, heart cells, epithelial cells, neuronal cells, and lung cells.

228. One or more cells of claim 208, wherein the cells are selected from precursor cells, progenitor cells, and differentiated cells of the hematopoietic lineage.

229. The bone marrow cell of claim 136, wherein the cell is selected from a stromal cell and a hematopoeitic cell.

230. The non-human animal of claim 202 that comprises an exogenous DNA sequence integrated into its DNA.

231. The non-human animal of claim 202, wherein the animal is a mouse.

232. The non-human animal of claim 202, wherein the non-human animal suffers from a disease, disorder, syndrome, or condition selected from abnormal growth, hormonal imbalance, early aging, abnormal bone formation, immune disorder disease, cancer, diabetes, skin disease, and CNS disease.

233. One or more isolated tissues derived from the non-human animal of claim 232.

234. One or more cells derived from the non-human animal of claim 232.

235. One or more cells of claim 234, wherein the cells are selected from stem cells, bone marrow cells, liver cells, pancreatic cells, skin cells, hematopoietic cells, kidney cells, heart cells, epithelial cells, neuronal cells, and lung cells.

236. One or more cells of claim 234, wherein the cells are selected from precursor cells, progenitor cells, and differentiated cells of the hematopoietic lineage.

237. One or more bone marrow cells of claim 235, wherein the cells are selected from stromal cells and hematopoeitic cells.

238. A method of determining gene function *in vivo* comprising the steps of

(a) providing a modified embryonic stem cell, wherein the modified embryonic stem cell comprises an introduced gene, wherein the introduced gene is a silencer and is present at a particular locus of the modified embryonic stem cell;

(b) introducing the modified embryonic stem cell into a blastocyst to form a modified blastocyst;

(c) implanting the modified blastocyst into an animal to produce a chimeric embryo, fetus or animal that expresses the introduced gene in more than one tissue; and

(d) determining or observing the effect of the introduced gene on the embryo, fetus, or animal.

239. The method of claim 238, wherein the silencer is an RNAi, antisense, or ribozyme.

240. One or more tissues derived from a chimeric embryo, fetus or animal of claim 238 or a progeny of such.

241. One or more cells derived from a chimeric embryo, fetus or animal of claim 238 or a progeny of such.

242. One or more cell lines derived from a chimeric embryo, fetus or animal of claim 238 or a progeny of such.